STUDIES IN THE LONGEVITY OF INSECTS.

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The investigation of the effect of temperature upon insects discussed below was undertaken at the suggestion of Professor C. W. Woodworth of the Entomological Department of the University of California in the fall of 1911. The purpose of the original problem was to obtain data on the length of the imago stage of the different orders of insects without food and under different temperature conditions.

The present article represents the extension of the original simple experiment to a more comprehensive study of the effects of temperature. The specific problems studied and the conclusions arrived at are as follows:

Part 1. Longevity as affected by different constant temperatures:

(a) is not correlated with systematic groups,
(b) differs inversely with these temperatures,
(c) is approximately proportional with these temperatures, and
(d) is primarily dependent upon physiological factors.

Part 2. Longevity as affected by exposure to two different temperatures:

(a) is increased when the temperature of first treatment is high or low, and
(b) is decreased when the temperature of first treatment is normal.

Part 3. Hibernation as affected by exposure to two different temperatures:

(a) is not brought to a close when the temperature of first treatment is normal and the temperature of second treatment is high,
(b) is brought to a close when the temperature of first treatment is low; is continued for from ten to twenty-one days and is followed by a second treatment at a high temperature, and
(c) is not brought to a close when the first treatment at a low temperature is continued for a period longer than three weeks and then followed by a second treatment at a high temperature.

METHODS.

The collecting of large numbers of insects which were brought alive from the field to the laboratory was greatly facilitated by the use of a net which Professor Woodworth invented some time ago. The advantage of this net is that insects may be procured by sweeping, even in damp weather, without the injuries which are usually the result of such collecting. The making of this net has been previously described by Mr. E. T. Cresson, Jr., who uses it continually for collecting small flies along the sand. Since, however, the net is not as widely known as its advantages deserve, another description will be in place:—

A strong piece of iron wire, three feet, eight inches long, is bent into a circle with a one foot diameter—the ends are then bent at right angles so as to lie adjacent and parallel to each other. These ends are inserted into the small end of a six inch ferrule and soldered fast. A short two foot handle will be found best for sweeping. The net consists of white muslin—a conical bag about eighteen inches deep. The tip is cut off where the circumference of the bag measures about three inches and is replaced by a small cloth bag four by six and a half inches. This small bag is sewed to the point at which the circumference of the large net is four inches, thus leaving a sleeve which hangs down into the small bag—this small bag will just hold a quarter pound paper bag. The sleeve of the large net fits into the paper bag. When filled from a minute's sweeping, the paper bag is pinched at the opening, taken out of the net and placed in a botanical can. Upon the return to the laboratory, the bag is opened at a well lighted window and the contents picked over for specimens.

When insects of one species were found in sufficient number to make it desirable to keep a number of them under observation as a unit, sets of capsules were bound together in tens as devised by Prof. Woodworth for his insecticide experiments. A piece of small iron wire two and a half inches long, sharpened at one end is thrust through the base of a gelatin quinine capsule so that the capsule is on the left of the wire with open end upward—a twist is made in the wire to hold the capsule on—then on the right side with open end in similar position, another

capsule is threaded upon the wire. In like manner, four more pairs of capsules are threaded on. The advantages are that the holes formed by the wire give ventilation and that the similarity of the position of the capsules makes a numbering system possible. The left hand first capsule bears the number of the set of ten written in ink on its face—the other capsules count up to ten in logical sequence from left to right towards the other end.

All insects were placed separately in capsules. If the insects were sufficiently duplicated in collecting, sets of capsules as above described were used. Otherwise the capsules were placed in envelopes, bearing data as to date of collecting, locality, temperature of collecting and temperature of treatment.

The envelopes or sets of capsules were placed on shallow wooden trays at different temperatures, room 62°F.—hot room 72°F or ice room 42°F. Each day the capsules were opened and examined, thus permitting a further change of air. Any insects that had died were removed and a number corresponding to the datum recorded was placed in the capsule. The specimens were generally simply classified to the family. The results are shown in the following table:—

Table 1. Longevity by Families.

Order	Family	Temperature										
		High 72° F.			Medium 62° F.				Low 42° F.			
		Longevity in Days.										
		Max	Ave.	Min.	Diff.	Max	Ave.	Min.	Diff.	Max	Ave.	Min.
Diptera	Mycetophilidæ	1	1	1						3	3	3
	Culicidæ	1	1	1						3	3	3
	Dolichopodidæ	3	3	3	6.0	15	9	3	6	15	15	15
	Syrphidæ					3	3	3				
	Acalyptræ	3	3	3						15	11	7
	Tachinidæ	3	3	3						15	15	15
	Sarcophagidæ					3	3	3				
	Cecidomyidæ	3	3	3		6	6	6				
	Anthomyidæ									3	3	3
	Muscidæ	7	1.6	1	.9	15	2.5	1	1.5	27	4	1
Coleoptera	Carabidæ	10	7	4						10	8	6
	Coccinellidæ	15	15	15						28	21.5	15
	Latridiidæ	15	9	4	5.5	25	14.5	4	7.7	33	22.2	15
	Malachiidæ	3	3	3								
	Tenebrionidæ					2	2	2		5	5	5
	Chrysomelidæ	8	8	8								
Hymenoptera	Curculionidæ	7	5.7	1	1.3	7	7	7	17.6	39	24.6	6
	Cynipidæ	15	5.2	1		8	5	3		15	7.3	1
	Proctotrypidæ					3	3	3		17	17	17
	Chalcididæ	15	5.2	1	.4	7	5.6	1	6.4	17	12	5
	Braconidæ	3	3	3		2	2	2		15	7	3
	Vespidæ	3	1.7	1						3	3	3
	Scoliidæ					3	3	3				
	Myrmicidæ	15	9.5	4						7	5	3
Hemiptera	Lygæidæ									7	7	7
	Anthocoridæ									7	7	7
	Capsidæ	6	2	1						7	7	7
	Membracidæ	3	3	3	.0	3	3	3	1.5	8	4.5	1
	Jassidæ	1	1	1	.0	1	1	1	1	3	2	1
	Aphidæ	3	3	3						15	9	3
	Aleurodidæ									15	15	15
	Coccidæ					15	15	15				
Orthoptera	Tettiginæ					5	5	5				
	Oedipodinæ									5	5	5
	Stenopelmatinæ	1	1	1		7	7	7		7	7	7
Lepidoptera	Nymphalidæ									37	37	37
	Papilionidæ									9	9	9
	Geometridæ									9	9	9
Neuroptera	Hemerobiidæ	3	3	3								

Table 2. Longevity by Orders.

Order	Number of Specimens	Temperature								
		High 72° F.			Medium 62°F.			Low 42° F.		
		Longevity in Days.								
		Max	Ave.	Min.	Max	Ave.	Min.	Max	Ave.	Min.
Diptera	303	15	1.8	1	15	2.5	1	27	4.2	1
Coleoptera	64	15	6.6	1	23	6.5	2	39	20.0	5
Hymenoptera	50	15	5	1	7	4.2	2	17	10.1	3
Hemiptera	24	6	2.5	1	15	5	1	15	6.7	1
Orthoptera	7	1	1	1	7	6	5	7	6.3	5
Lepidoptera	3	.....	.....	.....	.....	.....	.....	37	18	9
Aphaniptera	3	.....	4	.....	.....	3	.....	.....	1	.....
Thysanoptera	3	.....	15	.....	.....	15	.....	.....	15	.....
Neuroptera	2	.....	3	.....	.....	.....	.....	.....	17	.....
Insecta	359	15	4.8	1	23	6	1	39	10.9	1
Arachnida	26	15	8	1	17	13	3	15	8.8	3

In most cases the data are too few to be very significant as to individual groups but are sufficient to draw certain conclusions, viz:

1. That as regards longevity, the taxonomic divisions show little or no comparable variability. That is to say that the amount of variation in an individual species may be as great as the variability of the genus or family or even order making it appear that the average longevity of a large number of insects of one species would give the same results as the average of the same number of many species.

The following table in which the maximum, minimum and average longevity at each of the three temperatures is recorded for the order excluding the family with which it is compared, will show the above statement to be correct.

Table 3. Taxonomic Groups and Longevity.

Order	Family	Temperature.								
		High 72° F.			Medium 62°F.			Low 42° F.		
		Longevity in Days.								
		Max	Ave.	Min.	Max	Ave.	Min.	Max	Ave.	Min.
	Muscidæ...	3	2	1	15	6	3	15	8	3
Other Diptera.....	.....	7	1.6	1	15	2.5	1	27	4	1
	Curculionidæ...	15	9.9	4	25	6.1	2	33	17.3	5
Other Coleoptera...	.....	7	5.8	1	7	7	5	39	24.6	6
	Cynipidæ...	15	5	1	15	5.5	1	17	11.4	3
Other Hymenoptera	.....	15	5.2	1	8	5	3	15	7.3	1
	Chalcididæ	15	5	1	15	5.8	1	17	9.8	3
Other Hymenoptera	.....	15	5.2	1	7	5.6	1	17	12	5

2. That the longevity of insects in general is lengthened by a decrease in temperature and shortened by an increase in temperature (when these temperatures are between 42° and 72° F.)

Table 2 proves this to be true in all except two cases: (a) Coleoptera in general have a slight increase in longevity at high temperatures over that of the medium temperature. (b) Fleas in the three specimens tested show increase in length of life as the temperature increases. (c) (Arachnida have the greatest longevity at medium temperature).

3. That the difference in longevity of a species at different temperatures corresponds roughly to the difference in temperature. Table 4 shows that the greatest difference in length of life is between the longevity at Low and the longevity at Medium temperatures—this corresponds to the greater difference between Low 42° F. and Medium 62°F. as compared with the difference between Medium and High 72°F.

Table 4. Proportion Between Temperature and Length of Life.

Order	Family	Temperature				
		High 72° F.		Med. 62° F.		Low 42°F.
		Longevity in Days				
		Ave.	Diff.	Ave.	Diff.	Ave.
Diptera		1.78	.7	2.5	1.7	4.2
	Muscidæ	1.6	.9	2.5	1.5	4
Coleoptera		6.6	.1	6.5	13.5	20
	Latridiidæ	9	5.5	14.5	7.7	22.2
	Curculionidæ	5.7	1.3	.7	17.6	24.6
Insecta		4.8	1.2	6	4.9	10.9

The most important conclusion arrived at is that longevity is not correlated with systematic groups. Table 3 (Taxonomic Groups and Longevity) upon which this conclusion is based was compiled in each case from the family in which the greatest number of specimens had been included in the experiment. It is not probable that the greater variation in a family than in the average of the other families of that order as is apparent in the table, is due to any greater adaptability to temperature changes in that family than in the others. For a comparison of the maximum and minimum number of days that the representatives of the different families lived will show that individual variation within the family, in the majority of cases where a number of specimens of one species were used in the experiment, is as great as individual variation for the group. This great individual variation is probably due to the physiological conditions of the individual. For example, in the Capsidæ; of the five specimens of one species placed at a high temperature, all died in one day except one which moulted and lived for six days. Apparently the longevity in this case was due to individual physiological conditions and not to any inherent temperature adaptability. Such cases could be multiplied.

We may therefore come to another conclusion, viz:

4. That longevity at different temperatures is due to individual physiological conditions and that any attempt to determine the temperature longevity of the species would be confused by the variability of the results unless these physiological factors were brought into account.

It has been the general belief among entomologists that many insects of the orders Diptera, Lepidoptera and Hymenoptera in the imago stage take no food. Recent experiments (Doten 15) have shown that some parasitic Hymenoptera take food in the adult stage. Closer observations may prove this to be the case with many of the insects which are at present, thought to abstain from food. However, most insects do not feed after the eggs are fully developed. Whether or not, starvation is a factor in this experiment, must therefore be left undecided for the present.

PART 2.

EFFECTS OF EXPOSURE TO TWO DIFFERENT TEMPERATURES ON LONGEVITY.

It was found in Part 1 of these experiments that longevity varied greatly according to the physiological conditions of the individual—in order to obtain further data on the nature of these physiological conditions, the following experiment was performed:

It was thought probable that temperature could produce certain of these physiological conditions—therefore, an attempt was made to find if exposure to a certain temperature for a short time would result in a condition that would be evident in its influence on the longevity of the insect at a secondary and different temperature. The insects used as objects upon which to experiment were the larvæ of the very common oak tree moth (Phryganidia californica). The larvæ were placed separately in capsules, wired together in sets of tens as explained under "Method" in Part 1 of this paper. The sets of capsules were then placed in wooden trays at medium or room temperature at high or the temperature of a bacteriological incubator or at low, the temperature of an ice room, six by twelve by five feet. After two days' preparation at one of these temperatures, the larvæ were transferred to one of the other temperatures where they were kept until starvation resulted in death. The larvæ were examined each day and the date of death recorded.

"Experiment A" represents the results on one hundred young larvæ of the first brood of 1913. "Experiment B" represents the results with eighty-four older larvæ of the second brood of 1912.

Chart I records the results of these two experiments. The abscissa of each of the points marked with circles is the longevity of the larvæ at the constant temperature represented by the ordinate. Each arrow leaving one of these points runs to a point indicating in the same manner the longevity resulting from the treatment at the two temperatures.

Chart I shows that from any change in temperature there results an increased longevity of the larvæ, as follows:

Two Days Treatment at	Followed by	Results in	Longevity at constant
98° F.	58° F.	Same longevity as.....	58° F.
98° F.	68° F.	Increased longevity over.....	68° F.
68° F.	98° F.	Decreased longevity below.....	98° F.
68° F.	58° F.	Decreased longevity below.....	58° F.
58° F.	98° F.	Increased longevity over.....	98° F.
58° F.	68° F.	Increased longevity over.....	68° F.
82° F.	64° F.	Increased longevity over.....	64° F.
64° F.	82° F.	Decreased longevity below.....	82° F.
64° F.	46° F.	Increased longevity over.....	46° F.
46° F.	82° F.	Increased longevity over.....	82° F.
46° F.	64° F.	Increased longevity over.....	64° F.

The exceptions to this rule are in three cases: (a) where 68° F. is the preliminary two day treatment, there is a decrease, (b) where the change is from 98°F. to 58°F. there is no increase and (c) where 64°F. is followed by 82°F. there is a decrease in the longevity.

From the data recorded on Chart I, we may form two conclusions; (1) that the life of the larvæ of Phryganidia californica will be lengthened at any temperature (when starvation is a factor) by placing the insect for two days at either a high or a low temperature; (2) that the life of the larvae of Phryganidia californica will be shortened at any temperature (when starvation is a factor) by placing the insect for two day at a medium temperature.

The temperatures that have been found to have the characteristic effects described are: "High" 98-82°F.

Above 98°F. will probably have other characteristics as 98° F. already shows a transition in that it does not cause an increase in longevity when followed by a low temperature.

"Medium" 68-64°F.

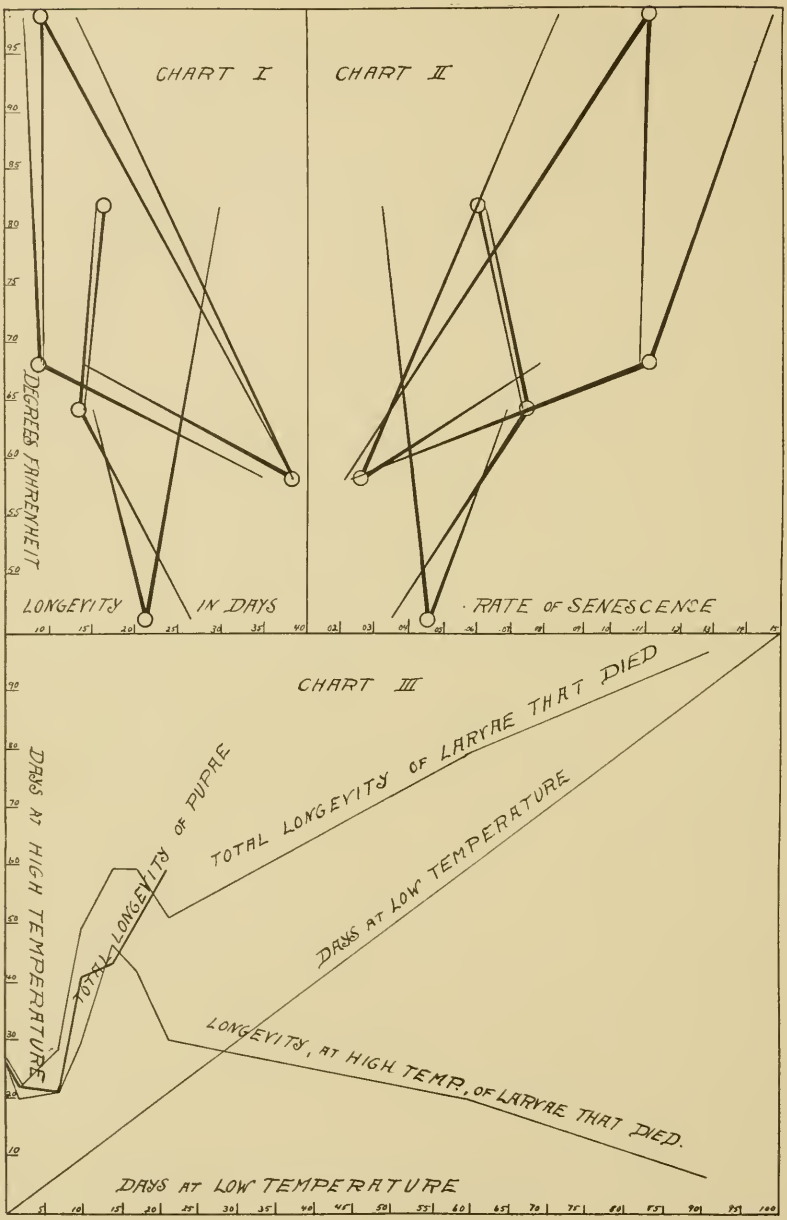
CHART I
CHART II
DEGREES FAHRENHEIT
LONGEVITY IN DAYS
RATE OF SENESCENCE
CHART III
DAYS AT HIGH TEMPERATURE
TOTAL LONGEVITY OF PUPAE
TOTAL LONGEVITY OF LARVAE THAT DIED
DAYS AT LOW TEMPERATURE
LONGEVITY, AT HIGH TEMP. OF LARVAE THAT DIED.
DAYS AT LOW TEMPERATURE

Between 64°F. and 58°F. there must be a temperature with another characteristic for 64°F. holds a transitory position in that it gives an increase in longevity when followed by a low temperature and a decrease in longevity when followed by a high temperature.

"Low" 58-46°F.

These temperatures show the characteristics ascribed to "Low Temperatures."

Since death by starvation is the end of the phase we are studying in this experiment, it was thought that probably a measure of the rate of growth would determine this rate of senescence. Sanderson recommends the following method of obtaining a temperature growth curve, viz: that a "definite valuation" be found "in relation to the accumulation of temperature necessary for any stage of growth" in the following manner: if, at a certain temperature, it requires x days to go through certain phases and this development be considered equal to one unit, then each day's growth at this temperature is equal to $(1 \div x)\%$. Using this method, the following tabulated growth valuations were found:

Table 5. Temperature Growth Valuations per Day.

Exp.	No. Days 1st. Temp	Temp.	Growth Value 1st Temp.	No. Days 2d Temp.	Temp.	Growth Value 2nd Temp.	Growth Value Total
A	9	High	.111 x 9	.....	.....	.....	1.0
	9	Med.	.111 x 9	.....	.....	.....	1.0
	38	Low	.023 x 38	.....	.....	.....	1.0
	2	Med.	.222	5.3	High	.147	1.0
	2	Med.	.222	33.1	Low	.0235	1.0
	2	High	.222	7.2	Med	.108	1.0
	2	High	.222	36	Low	.0216	1.0
	2	Low	.052	11.3	High	.0837	1.0
	2	Low	.052	12	Med.	.079	1.0
B	16.6	High	.0602x16.6	.....	.....	.....	1.0
	13.4	Med.	.0746x13.4	.....	.....	.....	1.0
	22	Low	.0454x22	.....	.....	.....	1.0
	2	Med.	.1492	24.5	Low	.0347	1.0
	2	Med.	.1492	13.6	High	.0625	1.0
	2	High	.1204	12	Med.	.0733	1.0
	2	Low	.0908	13.2	Med.	.0689	1 0
	2	Low	.0908	27.8	High	.0323	1.0

The irregularity of the results given in the next to the last column show that there is some other factor involved in the determination of the longevity of starving larvæ at different temperatures The most probable factor is the rate of metabolic processes for it is the most closely connected with temperature and nutrition, of any of the vital processes. Since the rate of growth and the rate of metabolism will determine how long the insect can live on the reserve material in its body. If the data of Table 5 is plotted in a similar manner to the data of Chart I, the graph on Chart II is obtained.

But if it is true that the rate of growth and of Metabolism determine the longevity it is necessary to bring another factor into consideration before we can explain why a two days treatment at a low temperature will decrease this rate when the insect is placed at a high temperature.

Growth changes in rate with advance in age but is not the process that results in death for while growth is due to the establishment of a constant relation between the nucleus and the cytoplasm and therefore must finally reach a stage where the growth is stopped, senescence always results in a decrease in weight which cannot be accounted for by any theories of growth according to Robertson (43, 44). Still a fall in the rate of metabolism accompanies old age—hence, we must conclude that there is another factor than growth that determines this rate of metabolism. It has been determined that the speed of the metabolic processes decreases with age—therefore, it may be determined by a measure of senescence. The progress of senescence has been defined variously by several investigators. Minot (35) basing his theories on certain truths, which others have used in supporting the theory that a nucleus can control but a limited mass of protoplasm (Sachs and Boveri), has measured the rate of senescence by growth. This has been shown to be improbable as before stated by Robertson and by Loeb (30) and Moore (36) who found that the temperature co-efficient of growth (2.8) is very different from the temperature co-efficient of longevity (1000).

Minot finds that senescence results from a gradual shifting of the ratio between nuclear and cytoplasmic substance (Kernplasma relationship) to the side of the cytoplasm and from the differentiation of the cells which accompany this change. This

differentiation, he claims, is irreversible. He therefore makes no provision for rejuvenation in the Metazoa. C. M. Child (10) has recently constructed a theory, which I will describe shortly, based on certain experiments and upon our present knowledge of the cell activities.

Cells go through two processes—one constructive and beneficial or life-giving, i. e. metabolism—the other destructive, katabolism. Both are necessary to life and a balance is maintained between them—when however, this equilibrium is upset in the direction of the katabolic processes, senescence is the result and finally death. The true measure of senescence then, may be taken to be inversely the rate of metabolism.

In the life processes, many compounds are formed which cannot be made in the laboratory without the use of great heat or chemicals which are incompatible with life. It is believed more and more generally that a study of the physical conditions of the life substance, protoplasm, would throw great light on these processes. Alsberg in his recent paper (2) on the mechanism of cell activities, has given a resumé of present day knowledge and conjecture on the subject.

The nature of protoplasm has been found to be similar to that of colloidal solutions and to emulsions. It is made up of substances that tend to concentrate at surfaces—this concentration and reduction of the size of the phase results in an enormous surface energy, which increases in immense proportion to the smallness or roundness of the surface of that phase or chemical locality. The very general composition of protoplasm, i. e., 80% water, 15-20% solid and 5% fats, would make its rigidity impossible were it not for some emulsified condition. It being a well known fact that emulsions often show great rigidity.

Since these substances have a tendency to form phases or localizations of chemico-physical conditions and since all these phases are in contact and all differ more or less in permeability, it is very possible that they act as a long series of interacting yet separate, semi-porous test tubes. A reaction may go to a certain stage then penetrate into the next phase and while being isolated and going through another reaction, may still influence the first phase—thus making it possible to complete a very complicated and apparently impossible chemical change.

Since the substances of this colloidal or emulsified solution have a tendency to collect at surfaces and when once out of solution (according to Loeb (31) are very difficult to bring into their former condition, permanent, more or less impermeable bars to the process of metabolism may be set up. These may be broken down by a change in the chemical process or a change even in their rate, due to exterior causes of temperature or food quantity.

Childs in some experiments on Planarians finds that the toxicity of alcohol which he uses as a measure of the rate of metabolism varies inversely with the age of the animal, i. e., metabolic processes are being lowered and katabolism is gaining the upper hand. He finds however, that rejuvenation is possible by a change in the rate of these metabolic processes.

Since metabolic processes are carried on through alveolar walls of phases in the protoplasm of the cell and since the longer this process of metabolism is carried on at the same rate and in the same chemical nature, the more permanent these walls become, a lowering of metabolic processes, i. e., senescence due to the establishment of alveolar walls which have through their permanency become bars to the action of metabolism, is the result. He finds however, that a change in these processes will result in an increased rate being possible for them. If an animal is starved for a short time and then fed, its ability to withstand the alcohol is greatly increased—this can be explained by the probability that the processes have gone on in spite of the lack of food and that the actual accumulation of cytoplasmic alveolar walls of obstruction have been destroyed and the cell thus brought into a younger stage of differentiation.

If the animal is starved for only a few days, this increased resistance is very small, upon again refeeding.

The rejuvenation has not gone on to as great an extent, therefore the resistance is less than that of the animal starved for a longer time. A similar result is obtained with animals that have been forced to regenerate parts—the larger the piece is that has been regenerated, the greater the increase in resistance to alcohol. In the case of regeneration, direct visible data has been given by Godlewski (19) showing that regeneration actually leads to a simplification of cells and a reverse process of cytomorphosis that Minot did not take into consideration in

the formulation of his theory. It was also found by Child (11) that the older a Planarian is, the more likely fission i. e., formation of a new individual from a part of the old is to take place. This is probably due to the greater isolation that the tail region of the animal has, because of the clogged condition of the cells as age advances.

An application of these results of Childs, Godlewski and the late experiments of Loeb (32) and Lillie on permeability of membranes will make possible an explanation of all the results of these experiments.

It must be remembered in the first place, and above all that one factor of the experiment was starvation—second, that the insects were placed first for two days at a preliminary temperature and then at a different temperature until they died. Since the result of starvation at a temperature is to clear the cell of cytoplasmic obstacles to a certain degree. The preliminary treatment of an insect with starvation at a temperature will determine to a great extent the results of treatment at a second and different temperature. On the accompanying Chart II, I have therefore plotted, the rates of senescence. They were obtained by finding the value of each day at a certain temperature for completion of a phase but since the end of this period was death, they may serve as the measure of the degree of senescence.

Since death will finally be the result of physiological senescence, due to lack of food, we must bear in mind the distinction between this and natural death which is the result of morphological senescence, the reverse of which is taking place in this case.

Reference to this chart then, will show the degree to which any treatment of temperature will result in combined morphological rejuvenation and physiological senescence. It will be seen:—

1. That preparation at a high or low temperature will result in a combination of physiological oldness and morphological youngness which will make the insect more liable to live, if it be placed in any other temperature, longer than if it had been living constantly at this secondary temperature.

2. That preparation at a medium temperature will render the insect older, both morphologically and physiologically and

therefore less liable to live, if it be placed at any other temperature, longer than if it has been living constantly at this secondary temperature.

The rapid starvation at the high temperature has morphologically rejuvenated the insect but has rendered it physiologically old. This slowing down and probably also change in function has rejuvenated and removed the cytoplasmic obstacles while morphological age, due to destruction of reserve products, has gone on to a less extent than at the high temperature. At medium temperatures, there is no change in rate nor a great enough degree of starvation to remove these inactive substances—therefore the cell is not rejuvenated morphologically and is physiologically old. In other words, the insect is older than the insects prepared by either of the other two methods.

PART 3.

EFFECTS OF EXPOSURE TO TWO DIFFERENT TEMPERATURES ON HIBERNATING INSECTS.

In part 2 of this article, certain studies of the effects of temperature upon the longevity of starving insects were made. In this part, I propose to further substantiate the statements made by the results obtained from certain experiments on the hibernating brood of the Codling moth larvæ (Carpocapsa pomonella L.)

The experiment was started with larvæ collected from windfall apples gathered under the trees and sent by the courtesy of Mr. Frank Perry of Sebastapol, Sonoma County, California, where the insects were collected. These insects were taken in the late part of July, 1913, and many of them pupated. Believing these to be of the earliest second brood, the experiment was abandoned and begun over again with larvæ that were collected in the cocoon—all the two hundred and fifty larvæ of the second experiment were collected in one mass of cocoons under a packing house. There could be no doubt then as to their hibernating condition and as to the similarity of their exposure to temperature, humidity and disease.

The larvæ were handled in the following manner: the cocoons were opened and two larvæ dropped into each clean test tube which was then plugged with cotton. The test tubes

were mixed to avoid the possibility of having a set of larvæ from the same part of the mass of cocoons. The test tubes were placed in round paste board boxes which gave room for seven of them and insured perfect darkness—a long strip of paper was placed in the box upon which was kept a complete record of the temperature treatment.

The insects were kept at three temperatures—room temperature as a check, low temperature in a refrigerator, usually about 43° F., or high temperature 86 to 96° F., maintained by an electric light.

The first experiment performed was to place a set of test tubes at the high temperature—the larvæ of this experiment all died in twenty-six days except one, which pupated, but did not hatch. An attempt was then made to bring the larvæ out of their hibernating condition by first chilling and then heating. Sets were placed in the refrigerator for varying lengths of time —it was found that an exposure to cold of from seven to fourteen days greatly lengthened the life of the larvæ and raised the percentage of pupation and of hatch. This percentage is much higher than that obtained by heat without previous chilling or by exposure to room temperature as in the check.

After fourteen days it will be seen by reference to Chart I that the longevity does not increase and that no pupation occurs. Four conclusions can be drawn from Chart I—

1. That pupation of hibernating Codling moth larvæ is not usually brought about by heat.

2. That exposure of these larvæ to a low temperature for from one to two weeks followed by heat results in pupation, hatch and increase in longevity of those larvæ which do not pupate.

3. That after twenty-one days exposure to low temperature, heat does not result in pupation nor is the longevity increased.

4. The number of days which the larvæ that die, live at the high temperature, is approximately equal to the total number of days, the other larvæ take to pupate.

In order to arrive at some conclusion about these experiments, first, let us consider the nature of hibernation. Hibernation takes place in many forms of insects, fish, Amphibia, Mollusks, birds, Mammals and even in man. Peasants of Russia, according to Cleghorn (12–13) with the approach of

famine, build a fire in a huge stove which serves as a resting place and lying upon this, keep as quiet and warm as possible and thus reduce their need of food. Among the Mammals, the marmot has been the most studied of the hibernating forms. Cleghorn lists a number of animals that hibernate—he states that bats of different species hibernate at different times of the year—that when disturbed for a time, they breathe almost normally and then again, the respiration goes down almost to zero. If awakened suddenly by great heat, death always ensues. He says that bears are as fat after hibernation as when they go into it in the fall and that female bears even raise their young while not obtaining any food and still show very little change in condition. Bears and badgers of the North do not go into any true state of hibernation but sleep lightly through the winter. The black bear, however, is aroused with difficulty from the winter sleep—the woodchuck of Canada, the European hedgehog, chipmunks and ground squirrels, all hibernate. Frogs hibernate in mud at the bottom of pools and if awakened by warmth can remain much longer under water without being drowned than during the active season. Some fish survive long draughts by burial in the mud. Baker (5) states that during some seasons of draught, Lymnæidæ bury themselves and form an epiphragm inside the outer lip as is common with Helix during hibernation and æstivation. Plants have a similar phenomenon also known as hibernation which is closely connected with lowering of temperature and shows itself in the decreased rate of the metabolic processes.

The physiology of hibernation has best been studied by Bellion in the European edible snail (1' escargot). Bellion (6) finds that the moisture content of the air and not temperature is the essential external factor of hibernation—when the moisture content is low, and epiphragm is formed in spite of low or high temperature and the snail is plunged into a condition of lethargy. If moisture content is high, no epiphragm is formed and activity is at its height even at a low temperature. Carbon dioxide content of the tissues increases towards the end of hibernation while the oxygen content diminishes in proportion. Dubois (16) has found in the marmot that when carbon dioxide is present in a certain proportion in the blood, torpor sets in. At moment of awakening, carbon dioxide is high—it is very probable that the carbon dioxide and rehydration

awaken the snail, as the carbon dioxide and dehydration plunges it into sleep. The amount is the essential to sleep or to awakening. Janichen (25) believes that the theory of autonarcosis of carbon dioxide should be held for all cold blooded animals.

The histological changes of hibernation have been studied in the hedgehog by Carlier (9). Plasma cells with deeply staining granules and with lightly staining nuclei are present in great numbers in the base of the tongue—they have the appearance of overfed cells although the fact that they are not found far into the digestive tract, seems, he states, to contradict this appearance. During hibernation the granulations disappear and the tissues of the tongue are less stainable. Numbers of the wandering white blood corpuscles are destroyed by macrophags and their number is recuperated all during hibernation by karyokenetic division in the lymph glands. During this period, some liver cells increase in size followed by an enlargement of the nucleus until the latter, having overstretched the nuclear network, ruptures and disappears—this Carlier believes to be the natural death of the cell.

Insects usually hibernate towards the end of summer when the temperature is falling but they are also known to go into this condition even though placed at a high temperature. Tower (53) found in his experiments with the potato beetle that he was unable under any laboratory conditions of high temperature to bring the beetles into hibernation at an unusual time. Sanderson (47) found that tent caterpillar eggs will not hatch if placed in a green house before being exposed to low temperature, while those which stay out of doors until the temperature falls will hatch rapidly at green house temperature. Merrifield (34) concluded from his experiments with seasonal dimorphism that there is probably a strong tendency for individuals to take either the winter or the summer form in spite of all temperature treatments.

Weismann found that summer forms could be obtained in winter (55), by chilling a pupa and then subjecting it to heat, while on the other hand, if the pupæ were put immediately at a high temperature, they did not hatch until summer. There is further data to show that low temperature is in many cases not the only factor in hibernation. Foster (*) states that of seven-

* Life History of the Codling Moth, U. S. D. A. Bur. Ent. Bul. 97, Part 2, Foster.

ty-eight Codling moth larvæ collected on July 17, at Walnut Creek, Cal., thirty-eight pupated, twenty hibernated as larvae and twenty-eight died. The temperature at Walnut Creek during July in 1909 actually increased three degrees over the mean temperature of June. Most larvæ of the second brood leave the fruit by the first of September and ninety-five per cent. hibernate as larvæ—yet the temperature in September is 3.3° F. higher than the temperature of June. According to Simpson (52), at Grand Junction, Colo., of 33 Codling moth larvæ collected July 16-23, 1900, but one hibernated while of 192 collected from August 30 to September 4, 192 hibernated. The mean temperature of June was 63.3°F. of August 67.8°F. and of September 61.7°. Yet the percentage of larvæ that hibernated had gradually increased from June to September.

Sanderson (47) finds that some Lepidoptera of the North when introduced into the South, do not have an increased number of broods as would be expected nor do southern forms have more than the one hibernating period, which is common to them in their warmer clime when introduced into the North. He bases this statement on the fact that the following insects have but one generation in the South: tent caterpillar, peach borer, plum curculio, canker-worm, gypsy-moth, brown tail moth, and insects effecting native trees, all of which are indigenous to the North. Newell (39) claims that the cotton-boll weavil enters hibernation after the first hard freeze and not due to a mean average temperature of 60° F. or even of 43° F. This is contradicted by Sanderson (46) who claims that weevils hibernate when the average temperature falls below 60° F. Hunter and Hinds (24) agree with Newell in saying that hibernation begins after the first hard frost—though if the insect be deprived of food, it will go into hibernation when the mean average temperature is below 60° F.; at a temperature of 60 to 65° F. however the adults will starve.

Moisture may also be a controlling factor of hibernation as has been shown in the case of the snail in æstivation and hibernation and also in the case of æstivation in the fish and in the Lymnæidæ.

Frogs also go into æstivation during summer as do plants and probably all animal life in arid countries. Loeb (31) points out that lack of water may act similarly to a low temperature—this may account, he says, for the fact that seeds can be kept

alive for so long. The effect of ether on plants is similar to hibernation and since the action of ether is probably a drying, one, this may throw light on the importance of moisture in hibernation. Hunter (24) has found that dryness is desirable for hibernation—he finds that more weevils die during hibernation from exposure to moisture than from cold, on the other hand, high temperature and moisture are the best conditions for weevil larvæ to develop. Sanderson quotes Tower as keeping potato beetles in hibernation for eighteen months in a dry atmosphere. Immediately when placed at a normal humidity, they immerge from hibernation. Donaldson (14) finds that frogs differ in the rate of reabsorbing water during summer and hibernation—it being more rapid in the former—he also finds that the water content of the spinal cord varies with the season—during the growth period (May 30 to July 1) it is high and gradually diminishes towards the end of the season. Rulot (45) has found that during hibernation, the production of metabolic water sometimes falls to zero in the bat. Hatai (21) has found that the effect of partial starvation on the nervous system is to decrease the percentage of water by 24 per cent. upon returning to normal diet, the water content is found to be higher than in the check. Abbe (1) has found that soaking seeds in water before planting accelerates germination but that germination is greatest in dry soil.

Tower states that during hibernation, the cells take on a definite appearance due to loss of water, being shrunken and flattened. In all cells, the protoplasm takes on a colloidal granular appearance which is retained throughout the whole period. The nuclei have an extremely vegetative appearance—it often being impossible to show the presence of chromatin in cells which later will have abundant and active chromatic conditions. There is a twenty-seven per cent. loss of weight due to the emptying of the malphigian tubules of a red fluid and a three per cent. loss of weight due to the emptying of the alimentary canal that takes place just before hibernation in the potato beetle.

Tower believes that this lowering in water content makes the maximum and minimum at which protoplasm can survive change in temperature in either direction, greater. Upon emergence from hibernation the reverse of the process of preparation for hibernation takes place—there is a rapid gain of

water—the cytoplasm becomes more watery, vacuoles appear, the cells become larger and more turgid and the chromatic elements stain deeply and increase in size, thus presenting all the signs of intense activity. The preparation in the potato beetle for æstivation is similar to that of hibernation—the animal remaining underground until first rains. Tower states here that the reduction of water gives an increased capability of meeting higher temperatures.

Hibernation usually follows a period of great feeding—whether this is what makes hibernation possible or whether it is the controlling factor of hibernation or not is unknown. In the marmot, there is a definite storage gland called the hibernation gland and Cleghorn includes in his definition of hibernation, the formation of reserve fat to be used during that period. In the potato beetle, the great period of feeding takes place before hibernation and æstivation (a little less in the latter) this oversupply of food is stored up in the fat body and is used to a certain extent during hibernation for there is a decrease in weight of the insect during that period. The spermophile and the marmot according to Cleghorn go into hibernation immediately after having laid up the last layer of fat. This occurs at a period when their food is most plentiful. The frog according to Holmes (23) goes into hibernation immediately after a period of great feeding. There is some evidence that overfeeding takes place just before hibernation, in the Codling moth for example: Hammar (20) has found that the feeding period of the larvæ of the first brood (transforming directly into pupæ) lasted 24.7 days while that of the first brood which hibernated lasted 28.9 days and the whole second brood (hibernating) 34.2 days. In the next year (1911) he found that the first brood which was to transform had a feeding period of 21.2 days while that part of the first brood that was to over-winter as larvæ fed for 28.2 days. Jones and Davidson (28) find that the second brood feeds twenty days longer than the first and at a higher mean temperature. Jenne (26) finds in like manner that the over-wintering brood of larvæ fed a longer time (.8 of a day) than the transforming brood.

Morgulis (38) has found that during hibernation, the nucleus is nourished by the cell—during starvation on the contrary, the nucleus at first loses volume rapidly though it remains more or less unaffected after it has attained a certain

minimum size. It is possible that by diminishing the volumne it increases its absorbing capacity. Hibernation is also unlike starvation in its characteristic quiescence, for animals when starved are very active. In hibernation also,there is no regeneration of tissues while in starvation this often occurs.

Hibernation seems to have a close connection with the maturation of the reproductive organs. Tower has found that those potato beetles that have gained sexual maturity, do not succeed in passing through the hibernating period successfully. Sexual maturity is seldom gained before hibernation in the second brood of this insect. This activity is greatest immediately following hibernation. He finds that the germ cells remain in the female as oocytes during hibernation and develop rapidly after hibernation. There are two generations in all climates—it would be supposed, Tower says that at high temperatures, breeding would go on continually but every alternating brood has a rest period before breeding goes on—this rest period is æstivation or hibernation depending on climate. All grape leafhoppers that have reached sexual maturity are unable to pass through the period of hibernation successfully—only the very immature males and females live through the winter to produce the next brood (according to Johnson 27).

Morgulis quotes the case of the Rhein salmon which makes a sojourn of from six to nine and a half months in the Rhein, remaining without food, developing in the meanwhile, its sexual elements at the expense of fat and proteids accumulated before hand. Holmes states that the period of great feeding preceding hibernation supplies food for that period and for the development of the reproductive organs which are to come into full activity immediately after hibernation. Hibernating insects seldom arrive at sexual maturity before this period is over. Newell found that the female cotton boll weevils which have hibernated continue to deposit eggs for a much longer time than the others. Morgulis claims that insufficient feeding effects the ovaries the most; since these organs seem to often develop during hibernation, it is very improbable that inanition takes place during this period. Loeb quotes Giard and Caullery as having found that a regressive metamorphosis occurs in Synascidians and that the animals hibernate in this condition. The muscles of the gills of these animals are decomposed in their

individual cells. The result is a formation of a parenchyma which consists of single cells and of cell aggregates resembling a morula. It is probable that a similar disintegration of parts takes place during hibernation and it is certain that it takes place during pupation. According to Sharp, when the larva of an insect has attained its full growth, many internal tissues disintegrate and rudimentary sex organs reabsorb the products of disintegration and with the other regenerative buds produce the perfect imago. On the contrary Jordan claims that the longer duration of the period of oviposition in the newt as compared with many other Amphibia may perhaps be correlated with the absence of the "fasting habit" (29).

The foremost essential factors of hibernation judging from the above observations seem to be temperature and moisture conditions, over-feeding and maturation of the reproductive organs. It is often stated that the loss of water makes it possible for the cell to withstand freezing temperature—for otherwise, as is claimed to be the case in plants (Vines 54) the ice crystals formed would rupture the cells. It is a known fact however that if cooled very slowly cells in which ice crystals have been formed, will again become normal. Tower and Sanderson state that the loss in water of the protoplasm makes it possible for this substance to stand greater variation in temperature for the concentration of salts makes the freezing point lower. But it is a known fact that the freezing point of sols is but sl ghtly lowered by an increase in the concentration of a salt dissolved. They also believe that it makes the protoplasm more able to withstand the high temperature but Loeb and Bachmetjew (3 and 4) have found that the point of coagulation of colloidal substances varies inversely with water content. This may account for the great killing of hibernating insects which Wright (56) ascribes to a rather warm winter.

Most animals that hibernate do so at a period just following great feeding and often at a time when their food is at its greatest abundance, as for example the cotton boll weevil, according to Sanderson (46). In some cases there is cytological evidence of overfeeding—for example, the overfed plasma cells in the hedgehog and the vegetative staining quality of the cells in the potato beetle and as I have found in the Codling moth larvæ. Overfeeding leads to increased number of molts or to hypermetamorphosis according to Sharp (51) who claims that

ecdysis is an extra excretory process. Quaintance and Brues (42) found that highly nutritive foods caused less molts but insufficient and disagreeable food resulted in more molts.

Sharp says that many hibernating larvæ have an extra molt. This may be either a sign of over-feeding or of feeding on some less nutritive substance in larger quantities.

I found in my first experiments with Codling moth larvæ in windfall apples that those larvæ which were about to hibernate remained inactive in the apple for some time (two days to a week) without eating before leaving the fruit to form a cocoon.

If it is granted that there is a condition of over-feeding in the larvæ before hibernating, it will be seen that there are many similarities between this stage and the condition before and during the molt. Before the molt, there is a period of great feeding—then a short period of quiescence, then the histolysis begins.

The process of histolysis is one of rejuvenation—in the second part of this paper, a résumé of the present day knowledge of the process of senescence was given—the most up-to-date and I think the best of these theories is the one advanced by Child. Child, basing his theory on the alveolar nature of protoplasm and on the nature of the metabolic processes and their tendency to lead to structural differentiation in the establishment of cytoplasmic alveolar walls, formulates the following law: "Senescence in nature consists physiologically in a decrease in the rate of metabolism and this is determined morphologically by the accumulation in the cell of structural obstacles to metabolism, e. g., decrease in the permeability, increase in density, accumulation of relatively inactive substances, etc. Rejuvenescence consists physiologically in an increase in the rate of metabolism and is brought about in nature by the removal in one way or another of the structural obstacles to metabolism." Since in the process of pupation, the tissues pass through a more or less complete process of histolysis which is aided by phagocytes, and new tissues often arising from germinal buds absorb this old material (Sharp, Packard (41) and Ganin (18), the cells of these tissues are probably less complex in their cytoplasm. Sharp says that the physiological conditions of the later larval life are different from those of the earlier

life, possibly as the direct result of a mere aggregation of matter—such a histolysis as above described, would reabsorb and redistribute this extra matter in such a way as to clear the cells of all inactive substances. During hibernation in the frog (Morgulis quotes Leonard) a similar process of histolysis and shifting of the nucleocytoplasmic relation in favor of the nucleus takes place. Without doubt, the cells are rejuvenated in the frog during hibernation—the case of Synascidians has already been stated. Lillie has found that fresh water Planarians if exposed to starvation, ultimately return to an embryonic form. These experiments have been confirmed by Schultz (50).

Childs found in his experiments on Planaria that starvation and regeneration both lead to rejuvenation—starvation differs from hibernation in that the life processes go on at a high rate in the former while they are sunk almost to zero in the latter. Starvation does not lead to the lowering of the water content as hibernation does, except in the nervous system. The conditions of the cells in the hibernating or in the starving insect are quite different. In the hibernating animal, the condition is one of overfeeding and probably of old age—that is, the accumulation of inactive substances in the cell is very great. In the starving animal on the other hand, the conditions are morphologically extremely young and physiologically old (underfed). Child compares cells in the overloaded condition to an ovum and the starved young cell to a spermatozoan. Loeb (32) has found that fertilization increases the permeability of membranes. The action of fertilization is the same as rejuvenation. A similar rejuvenation may take place by change in feeding as Calkins (8) has found to be true in his experiments with Paramœcium—where no conjugation took place if a change in feeding were made at the proper time. This agrees with Child's theory that rejuvenation can be brought about by a change in the chemical process of metabolism.

One characteristic of overfed Planarians according to Child is the physiological isolation of parts due to the overloaded condition of the cells with inactive bars to metabolism in the cytoplasm. This isolation leads to fission or to a senescence, i. e., a lowering of the rate of the metabolic processes. In Codling moth larvæ that are about to hibernate, I have found very similar conditions to exist—first, the vital processes are

at a low ebb—second, there is apparently a physiologically isolation of parts—this isolation is evident in the following ways: the larvæ often become entangled or bound by a thread of a spinning larva close by. The bonds which are thus tied about them become so tight that the insect is almost cut in two. I have often observed that the posterior half of the insect may have died from the effect of this isolation and decay set in while the anterior part may remain unaffected for many days. Disease also has been observed in these experiments to spread very slowly through the insect—this also can be accepted as evidence of the overfed and senescent condition of the larva which is about to hibernate. This has generally been found to be the case in hibernating mammals on exposure to disease. (Carlier and Dubois (17).

It seems probable then, that the overfed condition of the insect and the "old" state of the cell has reduced the permeability to a great degree and as a result, the rate of metabolic processes is greatly lowered. The loss of water probably results in the alveolar walls going out of solution and being cast out. In starving Planarians and in those which have undergone regeneration according to Nussbaum and Oxner (40), granules are present throughout the tissues. These granules are absorbed by phagocytes from the body wall—a similar process takes place in the potato beetle during hibernation, according to Tower and is characteristic of the process of histolysis, according to Henneguy (22).

During the molt, pupal period, and apparently in hibernating Codling moth larvæ (as I have observed in my experiments) these granules are very abundant.

From these considerations, it is possible to formulate certain working hypotheses which will serve as guides for further experimentation and consideration of which may throw further light on the nature of the processes of hibernation. These hypotheses are:

1. That temperature is but a single factor and not necessarily the controlling one in hibernation.
2. That hibernation is usually concomitant with overfeeding and may be a result of that condition or the result of accumulation of inactive substances in the cytoplasm of the cell due to feeding on innutritive food.

3. That the loss of water which is general in hibernation probably results in a discharge of insoluble alveolar cytoplasmic structures which have accumulated and produced this premature senility with an accompanying lowering of the rate of the metabolic processes.
4. That starvation during hibernation together with this loss of water may result in rejuvenation, when aided by histolysis, and in increased permeability.
5. That this rejuvenated condition and increased permeability will, if stimulated to activity by heat, permit pupation in Codling moth larvæ, which in this case is the termination of the hibernating condition.

If we remember that the temperature at which colloidal substances coagulate lowers with decrease in water content and that long exposure to cold may result in this decrease in water as well as exposure to high temperature and also the following observations of Bachmetjew, we can explain that the result of a long exposure to cold is the same as the result of a short exposure to heat and that the intensity of the cold, shortens the length of the period (Henneguy):

(a). The relation of the point of coagulation varies with the water content and the point of protoplasmic rigor is also lowered by hunger.

(b). Hunger lowers the critical point in direct proportion to the number of days of its duration.

(c). The intensity of cold shortens the time necessary for cold rigor.

The use of the hypotheses just outlined makes possible an explanation of the results of this experiment. If hibernating insects are placed at a high temperature directly, before being exposed to a low temperature, the characteristics of starvation rather than those of hibernation will set in—in other words, the nuclear material will decrease in greater proportion than the cytoplasmic material. On the other hand, if the insect is placed at a low temperature the characteristic enlargement in the nucleus at the expense of the cytoplasm and due to the low temperature, according to Boring (7), will take place.

With the lowering in the rate of metabolism, due to low temperature, the inactive conditions of the cells and their

enlargement in nuclear material is the ideal condition for disintegration. This has been shown to be the case in the liver cells of the hedgehog by Carlier.

In my experiments, I have found that the tissues of hibernating Codling moth larvæ show the presence of granular substances, immediately after the larvæ have been exposed to the low temperature. Probably these granules indicate cytoplasmic obstructions which due to the disintegration and inactivity of cells have been thus disposed of, leaving the cell in a rejuvenated condition. Tower found in the potato beetle these same granules present in hibernation and immediately after hibernation, a resumption of the activities of the cell, a loss of the vegetative unstaining quality and a more watery and less differentiated appearing condition of the cell. If the insects that are hibernating are exposed for increasing lengths of time to a low temperature and then placed at a high temperature, the tissues will have become rejuvenated and therefore with an increase in temperature, acceleration of the metabolic processes and of growth can take place.

However, if this exposure to cold is of too long a duration, either too much of the water content will have been lost and coagulation corresponding with permanent heat rigor, will set in at a lower temperature than after but a short exposure to cold or disintegration of tissues will have gone on to too great an extent.

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